

Figure 1. Covalent display

Double stranded DNA containing the coding sequence for P2-A and the coding sequences for a diverse population of polypeptides is transcribed and translated *in vitro* and due to the *cis*-activity of P2-A, the expressed polypeptides spontaneously and covalently associate with their own encoding DNA molecules through the interaction between P2-A and its recognition sequence which is contained within the P2-A gene. The covalent Protein-DNA complexes are then used in affinity selection protocols against a given target in order to identify individual genes that encode ligands to the target.

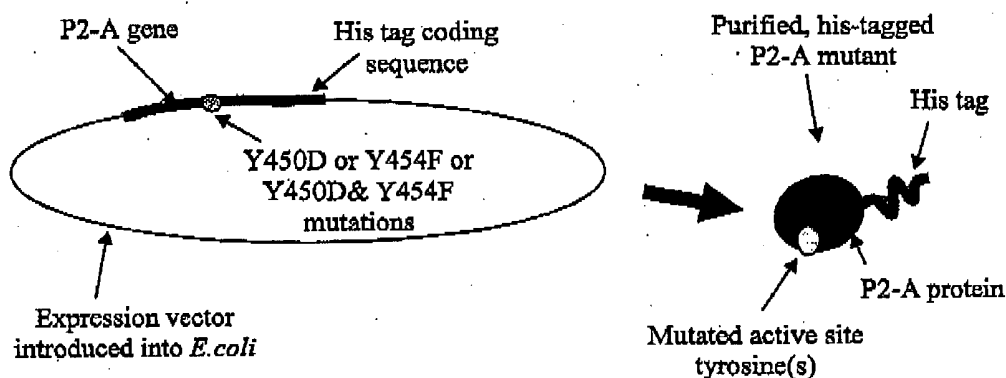


Figure 2. Liu's disclosure

P2-A wild type sequence, or P2-A genes in which either the putative active site tyr450, the putative active site tyr454, or both putative active site tyrosines were mutated, were cloned in frame with a hexa-histidine tag. The expressed polypeptides from these constructs formed inclusion bodies from which purified P2-A variants were isolated and used in biochemical analyses of the catalytic mechanism of P2-A. No attempt to isolate covalent complexes consisting of the P2-A gene and the expressed P2-A protein was made. No diverse population of P2-A-fused binding domains was made. No indication was made that the *cis* property of P2-A could be exploited for the establishment of a library screening method.

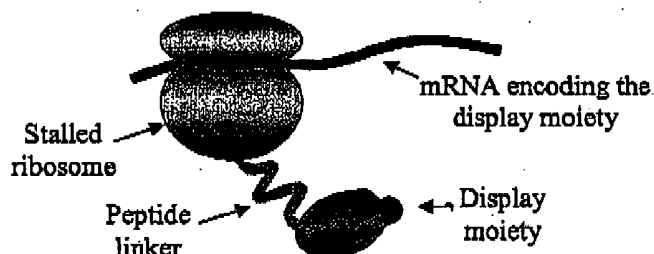


Figure 3. Basic Polysome Display

Double stranded DNA is transcribed and translated *in vitro*. By omitting to include stop codons at the end of the sequences encoding the display moiety and by carefully controlling divalent ion concentrations etc. a population of complexes form which consist of mRNA, stalled ribosome and nascent polypeptide. These complexes can be used for affinity selections against a given target in order to identify individual genes that encode ligands to the target.

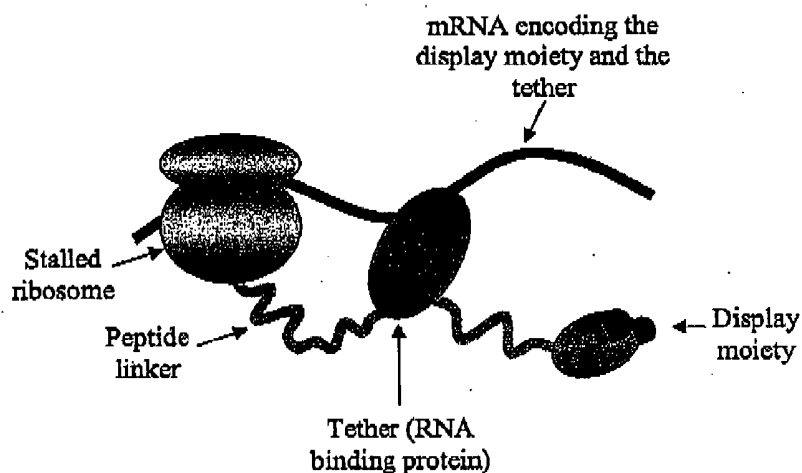


Figure 4. Mattheakis' tethered polysome display using a RNA binding protein tether.

Polysome display is operated as in figure 3 except that in addition to the coding sequence for the display moiety an additional coding sequence for a RNA binding protein is included in the mRNA template. By also including the recognition sequence of the RNA binding protein in the mRNA template the polysome complexes are stabilised by the interaction between the RNA binding protein and the mRNA template.

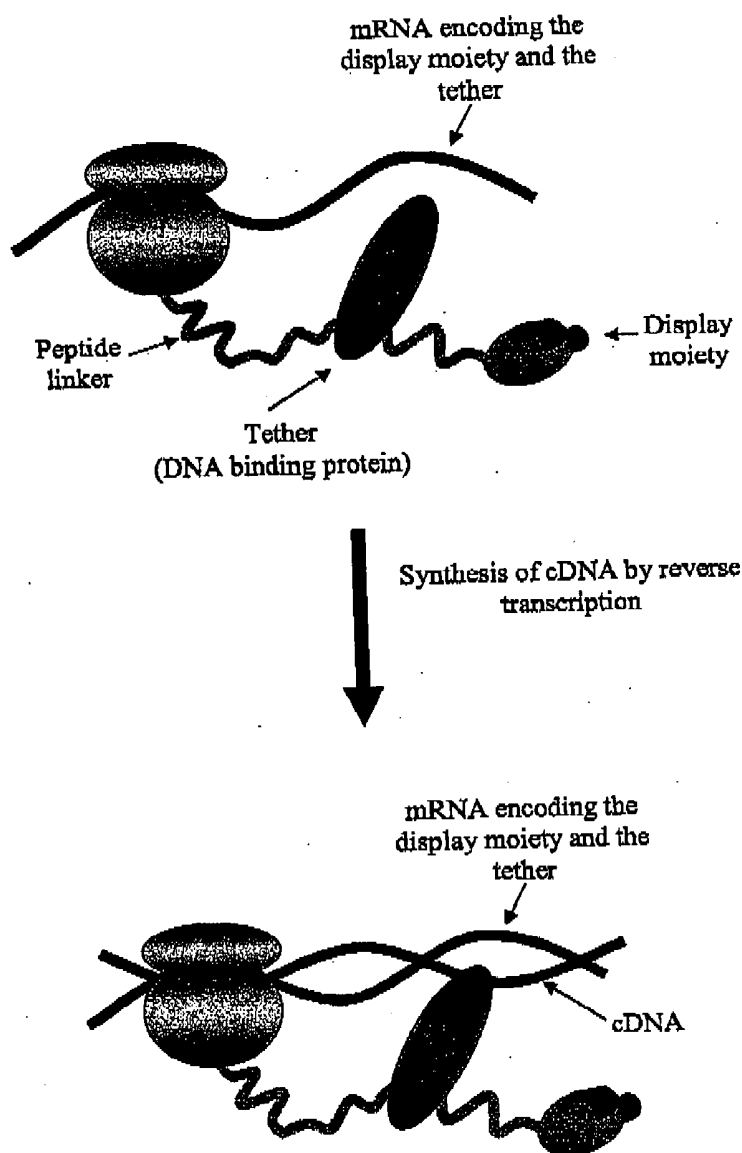


Figure 5. Mattheakis' tethered polysome display using a DNA binding protein tether. Polysome display is operated as in figure 3 except that in addition to the coding sequence for the display moiety an additional coding sequence for a DNA binding protein is included in the mRNA template. By also including the recognition sequence of the DNA binding protein in the mRNA template and by performing a reverse transcription step to produce cDNA after the formation of the polysome complexes, the polysome complexes are stabilised by the interaction between the DNA binding protein and the cDNA template.

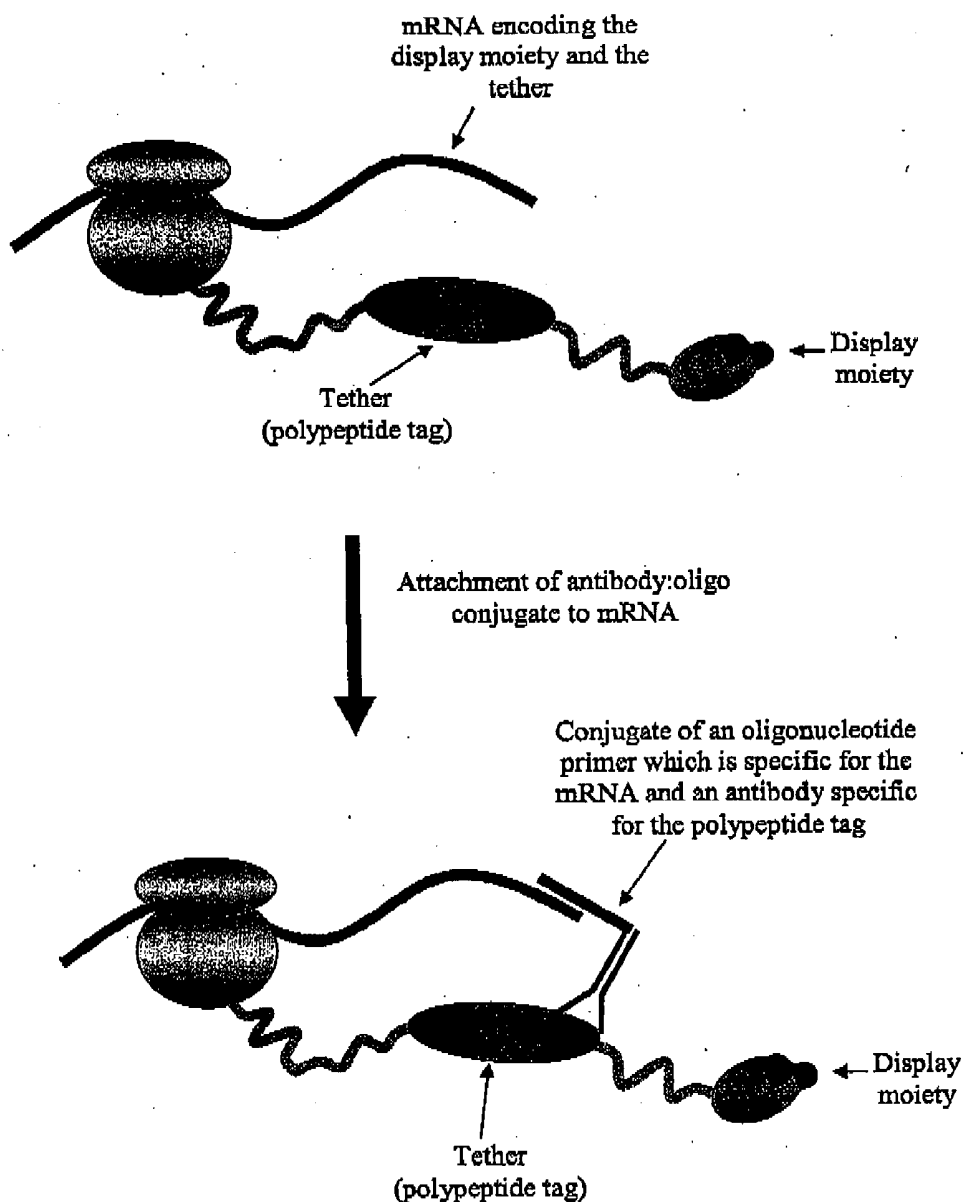


Figure 6. Mattheakis' tethered polysome display using a polypeptide tag. Polysome display is operated as in figure 3 except that in addition to the coding sequence for the display moiety an additional coding sequence for a polypeptide tag is included in the mRNA template. By attaching a ligand to the polypeptide tag (e.g. an antibody) to the mRNA template, either directly or indirectly (such as through hybridisation of a oligonucleotide tag-antibody conjugate as shown in the figure), the polysome complexes are stabilised by the interaction between the polypeptide tag and the attached antibody.